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Investigation of the Effect of Honey Bee Pollination for Apple (Malus sylvestris (L.)Mill.) on Fruit Yield, Seed Number and Seed Germination Capacity

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ABSTRACT
Apple is a self-incompatible fruit that is completely pollinated by cross pollination. Honey- bees are particularly effective in cross pollination. The aim of this study was to investigate the effect of pollination of apple by honey bees on the fruit yield, average number of seeds and germination capacity of the seeds. We observed that the average number of flowers in apple branches open to pollination ranged from 10.18 ± 0.53 to 278.43 ± 0.73 while the average number of flowers in apple branches close to pollination was between 8.07 ± 1.19 and 127.05 ± .057. The average duration of bee stay on apple flowers open to pollination was in the range of 3.66 ± 0.09 to 8.64 ± 0.07 seconds. The total number of fruits on the branches that were open to pollination was higher than those of close to pollination. Likewise, the average number of seeds on the branches open to pollination was higher than those of closed to pollination. We also observed that the average germination of seeds obtained from branches open to pollination was higher than the those of close to pollination.

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Elma (*Malus sylvestris* (L.) Mill.) Polinasyonunda Bal Arısı Kullanımının Meyve Verimi, Tohum Sayısı ve Tohum Çimlenme Kapasitesi Üzerine Etkisinin Araştırılması

ÖZET
Elma kendine uyuşmazlık sergileyen ve tamamen çapraz tozlaşma ile tozlaşan bir meyvedir. Bal arıları özellikle çapraz tozlamada etkin bir şekilde görev almaktadır. Bu çalışmanın amacı elma da bal arısı ile polinasyonun meyve verimi, ortalama tohum sayısı ve oluşan tohumların çimlenme kapasiteleri üzerine etkisinin araştırılmıştır. Çalışma sonucunda; polinasyona açık elma dallarında ki ortalama çiçek sayısının 10.18±0.53 ile 278.43±0.73 arasında değiştiği, polinasyona kapalı elma dallarında ki ortalama çiçek sayısının 8.07±1.19 ile 127.05±.057 aralığında olduğu, polinasyona açık elma dallarında ki arının bir çiçekte ortalama kalma süresinin 3.66±0.09 saniye ile 8.64±0.07 saniye aralığında olduğu tespit edilmiştir. Polinasyona açık ve kapalı elma dallarında ki meyve sayıları karşılaştırıldığında; polinasyona açık dallarda ki toplam meyve sayısının daha fazla olduğu, polinasyona açık dallarda ki ortalama tohum sayısı ile polinasyona kapalı dallarda ki ortalama tohum sayıları karşılaştırıldığında; polinasyona açık dallarda ki tohum sayısının ortalamasının polinasyona kapalı dallarda ki tohum sayısı ortalamasından daha fazla olduğu belirlenmiştir. Polinasyona açık dallardan elde edilen tohumların ortalama çimlenme miktarının, polinasyona kapalı elma dallarından elde edilen tohumların ortalama çimlenme miktarından daha fazla olduğu gözlenmiştir.

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INTRODUCTION

Apple (*Malus sylvestris* (L.) Mill.) is a pome fruit that is cultivated in many parts of the world because it is well adapted to different climate characteristics (Pereira-Lorenzo et al., 2009). All of the culture plants produce flower and seeds for their reproduction. Therefore, they need mostly foreign pollination to increase product quality and quantity. Honey bee is the best pollinator to perform foreign pollination. Honey bees need nectar and pollen to survive. They visit flowers to collect nectar and pollen that they necessitate. That way they carry out the pollination of many plant species which are became the nutrition of humans. In the absence of adequate pollination, seed production may not occur, and even if seed production occurs the end-products may have deformities due to poor development. As a result, the product value of fruits and vegetables decreases. Germination and growth characteristics of the seeds without pollination may not be at the desired level (Doğaroğlu, 1985; Kumova and Korkmaz, 1998; Korkmaz and Aydın, 1999). In pollination, insects, especially bees, have a very important role (Kumova and Korkmaz, 1998). Honey bees are active pollinators because they visit flowers of many plant species, move fast, and carry much pollen to the stigma of flowers. Honey bees are preferred as pollinators because they are easily replicated and transported, they are active except rainy, windy and very cold days and they are resistant against pesticides compared to other bees (Blanchet et al., 1991). The contribution of honey bees to the economy as pollinators is much greater than their contribution as honey and wax producers. Apple is a self-incompatible fruit that is completely pollinated by cross pollination. Honey bees are particularly effective in cross pollination (Stern et al., 2007). Apiculture is an important agrarian activity which contributes to the protection and development of environment, agriculture and forest products. The contribution of apiculture to crop production by pollination is 143 times higher than the sum of income from honey and wax production (Sıralı, 2003). The value of product obtained from the pollination is 50 times greater than that of honey produced annually (Crane, 1975). Although wind is considered as a pollinator, it is insufficient for pollination for many plants due to none consistence in pollination. Flowers need bees for pollination and bees need flowers to feed themselves (McGregor, 1976; Free, 1993). The most important factor for increasing productivity in crop production is an efficient pollination. The efficiency and continuity of many plant populations depends on seed production due to sufficient pollination (Korkmaz and Aydın, 1999). The effect of pollination and fertilization on increasing fruit yield and enhancing quality is essential. Bees play an important role in pollination. To provide proper pollination conditions, it is recommended to place 2 - 5 hives in 1 hectare garden area. Placing an appropriate number of bee-hives in orchards will ensure that the pollination conditions are kept at an optimum level (Soylu, 2003). Turkey has a great potential for fruit production and beekeeping. Turkey, having four climates and a rich flora of plants also makes this country has to have a wide genetic variation of bee races. Those factors create a suitable environment for beekeepers-fruit cultivation. Honey bees are an indispensable element of agriculture and must be used in pollination. This will in order to increase the quality and quantity in of agriculture agricultural products and will in order to make the beekeeping profession more attractive and the beekeepers more powerful (Kuvancı, 2009). When the importance of honey bees in pollination and their effective role in the quality and quantity of the products are fully understood, there will be a great improvement in agriculture. Was a result of this fact, we can conclude that besides honey, beeswax, pollen and royal jelly are additional products of honey bees. ion, the effective usage of honey-bees for pollination purposes will make an additional gain for both beekeepers and plant producers (Kumova and Korkmaz,1998). The aim objective of this study is was to investigate the effect of pollination of apple by honey bees on the fruit yield, average seed number of seeds and seed germination capacity. of the seeds.

MATERIALS and METHODS

This study was was carried conducted out in 2018, in Bingol province, in a six years old apple garden linked of to Bingol University in Bingol province, in 2018. by using a six years old apple tree. Cultural practices such as pruning, plant nutrition fertilizer, disease and pest control applications etc. were done applied as needed according to the standard. No pesticides were applied during the flowering period of the apple tree to provide a significant advantage for the honey bees pollination. Other plants with white flowers blooming at the same time with apple created a competition in terms of honey bee activity. Therefore, weeding was performed in the trial parcel. In order to reduce the impact of the direction on the pollinator, 6 branches were selected from different aspects side site of the tree. Three of the selected branches were closed to the honey-bee visit with tulle before flowering (during the period of pink bud). For each branch that was closed to the honey-bee visit, there was a branch left open in the same direction. The flowers on the branches that were closed to honey-bee pollination and on the branches that were left open were counted every two days and the average number of flowers on the branches was recorded. On each branch open to pollination 5 flowers were randomly selected and the average values of the honey bees visiting these flowers were calculated. The stay of the bee on a flower was calculated in seconds by taking

the average of the duration of the bees visiting that flower. During the flowering period, honey-bees were counted at 10:00 a.m. for ten minutes with two day intervals and the mean values of the number of bees visiting the flowers were found. At the end of the flowering period, the tulles covering the branches were collected. Fruits are counted to detect the effect of the application on fruit numbers. The life rate of fruits on the branches open and closed to honey-bee pollination was identified by counting the total number of fruits on the branches every 15 days until the harvest time. To analyze the effect of pollination on seed formation, three fruits were taken from every open and every closed branch, the seeds were counted and the average seed count values were calculated. The experiment was planned to have 3 branches in each repetition and the averages of these repetitions were found and the standard deviation values were calculated from these repetitions.

From every branch that was closed to honey-bee pollination and from every branch that was open to honey-bee pollination the healthy fruits are collected and 15 seeds were taken from each branch. The selected seeds were used for germination tests. Seeds obtained from the fruits grown on the branches closed to pollination were placed on petri dishes. Five pieces were put on every petri dish and repetitions are done triplicate. After 21 days the mean values were obtained for the germinating seeds on the petri dishes and the standard deviation values of the repeated experiments were calculated. The same process was also done for the seeds obtained from the fruits on the branches open to the pollination. Before the seeds were placed in petri dishes, blotting paper was placed into the petri dishes and the blotting paper was soaked with water to ensure germination of seeds. Petri dishes were kept at room temperature for germination and water was added when the blotting paper got dry.

RESULTS

The average number of flowers on the branches open and closed to honey-bee pollination, the average number of honey-bees visiting the randomly chosen 5 flowers on the apple branches open to pollination within 10 minutes and average duration of stay of honey bees on a flower (in seconds) are given in Table 1. The data was collected for 35 days, from April 17th 2018 to May 22nd 2018. The data was recorded every other day. The average number of flowers on apple branches open to pollination ranged from 10.18 ± 0.53 to 278.43 ± 0.73. The highest flowering was found on May 7th 2018 and the lowest flowering was on May 22nd 2018. The average number of flowers on the apple branches closed to pollination varied between 8.07 ± 1.19 and 127.05 ± 0.57. Also for those branches the highest flowering was on May 7th 2018 and the lowest flowering was on May 22nd 2018. When the average numbers of flowers on the apple branches open and closed to pollination were compared, we observed that the number of flowers was higher on the branches opened to honey bee pollination (Table 1).

The number of bees visiting the 5 randomly selected flowers on apple branches open to honey-bee pollination within 10 minutes varied from 7.74 ± 0.17 to 93.67 ± 0.37. While the number of bees visiting the flowers decreased at the beginning of flowering and at the end of flowering, their number rose during the period when the flowering increased. The number of honey bees visiting the flowers was highest on May 11th 2018 and their number was lowest on May 21th 2018 (Table 1).

The average length of stay of a bee on flowers on the apple branches open to honey bee pollination varied between 3.66 ± 0.09 and 8.64 ± 0.07 seconds. Towards the end of flowering the average stay time of the bees on a flower decreased. On the date of April 23th 2018 the average stay time of the bees on flowers reached its highest value and on May 19th 2018 the average length of stay on a flower descended to its lowest value (Table 1).

The change in the number of fruits on the apple branches open and closed to pollination until harvest time is shown in Table 2. Table 2 also presents the ratio of the difference of the total living fruit numbers on the branches open and closed to pollination to the total fruit number on the branches open to pollination as percentage. The counting of the fruits on the branches started on June 1st 2018. On October 15th 2018 the fruits on the branches were counted and the fruits were harvested. The counting of the fruits was done in 15 days intervals. The total number of fruits on apple branches open to honey-bee pollination was between 17 and 21, and the total number of fruits on the branches closed to pollination varied between 6 and 13. The fruit numbers were higher on the branches open to pollination. The date on which the total number of fruits on the branches was the highest was June 1st 2018, the date on which the number of fruits on the branches was the lowest was August 15th 2018. The total number of fruits did not change from this date on, until the time of harvest.

The ratio of the difference of the total number of living fruits on the branches open and closed to pollination to the number of fruits on the branches open to pollination varied between 38.09% and 68.42%. The total fruit loss rates on the branches open and closed to pollination were 19.04% and 53.84%, respectively (Table 2).

After harvest 3 fruits were randomly selected from each branch and the average seed numbers of the fruits grown on apple branches open and closed to honey-bee pollination were calculated (Table 3).

Table 1. The average flower number on the apple branches open and closed to honey bee pollination, the average honey bee number visiting 5 randomly chosen flowers within 10 minutes on the apple branches open to pollination and the average stay time of honey bees on a flower (in seconds)

Date	Time	Average flower number on the branches open to pollination	Average flower number on the branches closed to pollination	Average bee number on the branches open to pollination (10 minutes)	Residence time of bees on a flower open to pollination (second)
17/04/2018	10.00	60.13±1.03	40.27±0.63	17.54±0.05	7.03±0.06
19/04/2018	10.00	62.25±0.14	43.18±0.72	15.23±0.07	6.16±0.08
21/04/2018	10.00	74.06±0.68	56.25±0.96	24.61±0.12	7.14±0.03
23/04/2018	10.00	87.48±0.74	61.46±0.12	32.43±0.13	8.64±0.07
25/04/2018	10.00	108.54±0.41	78.17±0.58	36.26±0.16	7.06±0.12
27/04/2018	10.00	117.37±0.78	82.54±0.34	45.34±0.78	7.46±0.01
29/04/2018	10.00	145.65±1.52	87.64±1.06	56.67±0.36	8.13±0.03
01/05/2018	10.00	167.87±0.97	96.43±1.14	65.48±0.62	5.66±0.11
03/05/2018	10.00	210.79±0.63	101.74±0.87	72.21±0.23	7.13±0.14
05/05/2018	10.00	258.24±0.54	108.78±0.21	84.43±0.46	6.66±0.09
07/05/2018	10.00	278.43±0.73	127.05±0.57	78.19±0.91	6.03±0.05
09/05/2018	10.00	270.61±0.56	113.71±0.83	80.45±0.54	5.16±0.01
11/05/2018	10.00	275.76±1.07	109.32±1.14	93.67±0.37	7.66±0.07
13/05/2018	10.00	210.51±0.36	101.15±1.26	65.27±0.51	5.19±0.15
15/05/2018	10.00	185.18±0.69	97.56±0.45	24.46±0.19	7.26±0.04
17/05/2018	10.00	160.37±0.26	89.82±0.68	32.34±0.45	5.46±0.01
19/05/2018	10.00	88.07±0.17	51.08±0.39	14.18±0.32	3.66±0.09
21/05/2018	10.00	30.29±0.34	14.16±0.69	7.74±0.17	5.13±0.16
22/05/2018	10.00	10.18±0.53	8.07±1.19	9.15±0.09	5.03±0.08

±= Refers to the standard deviation value obtained from at least three repetitions

Table 2. The change of fruit numbers on the apple branches open and closed to honey-bee pollination until harvest

Date	Total fruit number on the branches open to pollination	Total fruit number on the branches closed to pollination	The ratio of the difference of the fruit numbers between the branches open and closed to pollination to the number of fruits on the branches open to pollination (%)
01/06/2018	21	13	38.09
15/06/2018	21	11	47.61
01/07/2018	19	7	63.15
15/07/2018	19	6	68.42
01/08/2018	19	6	68.42
15/08/2018	17	6	64.70
01/09/2018	17	6	64.70
15/09/2018	17	6	64.70
01/10/2018	17	6	64.70
15/10/2018	17	6	64.70
Loss at the end of harvest (%)	%19.04 wastage	%53.84 wastage	

Table 3. The average seed number on the branches open and closed to honey bee pollination

Apple branches used in the study	Average seed number on the branches open to pollination	Average seed number on the branches closed to pollination	Ratio of average seed number difference between the branches open and closed to pollination to average number of seeds on the branches open to pollination (%)
1. Branch	7.67±0.16	3.26±1.06	57.49
2. Branch	6.89±0.24	4.19±1.15	39.18
3.Branch	7.46±0.37	3.74±1.34	49.86

±= Refers to the standard deviation value obtained from at least three repetitions

The average seed numbers of the fruits on the branches open to pollination ranged between 6.89 ± 0.24 and 7.67 ± 0.16 and the average seed numbers of the fruits on the branches closed to pollination varied between 3.26±1.06 and 4.19±1.15. The average seed numbers of the branches open to pollination were higher than those of closed to pollination.

The ratio of the difference of the average seed numbers between the branches open and closed to pollination to the average seed numbers of the branches open to pollination was between 39.18% and 57.49% (Table 3).

The average germination amounts of seeds obtained from healthy fruits grown on the apple branches open and closed to honey-bee pollination is given in Table 4. The average germination of the seeds from the branches open to pollination was between 4.75 ± 1.46 and 5.65 ± 0.96 and the average germination amount of the seeds from the branches closed to pollination was between 1.98±1.03 and 2.07±1.64. The seed germination obtained from the branches open to pollination was higher than those of closed to pollination. The ratio of the difference between the average germination of the seeds obtained from the branches open and closed to pollination to the average germination amount of the seeds obtained from the branches open to pollination was between %52.21 and %63.36 (Table 4).

Table 4. The average germination amount of the seeds obtained from the healthy fruits on the branches open and closed to honey-bee pollination

Apple branches used in the study	The average germination amount of the seeds obtained from the branches open to pollination	The average germination amount of the seeds obtained from the branches closed to pollination	The ratio of the average germination difference of seeds between the branches open and closed to pollination to the average germination difference of the seeds from the branches open to pollination (%)
1. Branch	5.05±1.89	1.98±1.03	60.79
2. Branch	4.75±1.46	2.27±1.34	52.21
3. Branch	5.65±0.96	2.07±1.64	63.36

±= Refers to the standard deviation value obtained from at least three repetitions.

DISCUSSION

Pollination and fertilization events are of great importance for the fruit formation in pome fruit species such as apple. Pollination is the most important factor causing the yield increase (McGregor, 1976; Free, 1993). Lack of pollination causes a decrease in yield production (Oronje et al., 2012). Pollination is essential in the production of cultivated plants. If there is sufficient pollination, there will be an increase of 45-50% in sunflower, 50-60% in clover species, apple and pear, 75-90 % in cucumber, 95-100% in melon and watermelon and 35-40% in tomato, trefoil and vetch (Mel'nichenko and Khalifman, 1976; Mel'nichenko, 1977). In our study, the total number of apples on the branches closed to honey-bee pollination was lower than those of open to pollination, supporting the data in the literature.

The studies conducted with fruits with multiple cores like strawberry, grapes, tomato, kiwi and cucurbit discovered that the multitude of bee visits enabled seed numbers to be at adequate levels, fruits to be fine shaped and to have an intense taste and aroma (Woyke and Bronikowska, 1984; Blanchet et al., 1991; Cervancia and Bergonia, 1991; Goodwin et al., 1991; Shrivastava and Shrivastava, 1991; Svensson, 1991; Banda and Paxton, 1991; Morse and Calderone, 2000). In our study, the average seed number from the branches closed to pollination was lower than those of open to pollination. This result is consistent with the data in the literature.

Apple is a self-incompatible fruit that needs pollination to obtain high quality products. In the absence of pollination, the fruits shapes deteriorate and fall before developing fully as a result of some seed sketches. In a study using Granny Smith and Jersey Mac apple varieties grafted onto an M9 apple tree, it was found that honey bee activities resulted in a significant increase in yield and quality (Canverdi, 2016). In another study conducted in apple orchards in England, it was reported that the use of honey bees in the pollination increased the quality of fruit, regulated the level of minerals and caused an increase in production and quality (Garratt et al., 2014). In this study, we have shown that honey bee pollination had a significant effect on the average number of fruits and average seed production in apple.

In a study on seed production and quality of culture onion variety of Valencia, the authors reported that honey-bees had a nutritional search behavior between 8:15 a.m. and 16:30 p.m., but the most intense nutritional search activity was between 11:00 a.m. and 12:00 p.m. (Yücel and Duman, 2005). In our study, we chose 10:00 a.m. to observe the visits and the duration of stay on flowers for the honey-bees. We chose the time when bees work most intensively to increase the reliability of our findings.

A study on the effect of pollination on the germination of *Lasthenia california* seeds showed that pollination increased seed germination capacity and effected germination quality positively (Hendrickson et al., 2018). In our study, we observed that the average germination of apple seeds was higher when honey bee pollination was present.

As a result of this study, we conclude that there is a significant relationship between bees and plant pollination. To obtain a high quality and quantity of products and to increase the amount of seed production and germination, bee colonies need to be placed near to cultivation sites.

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